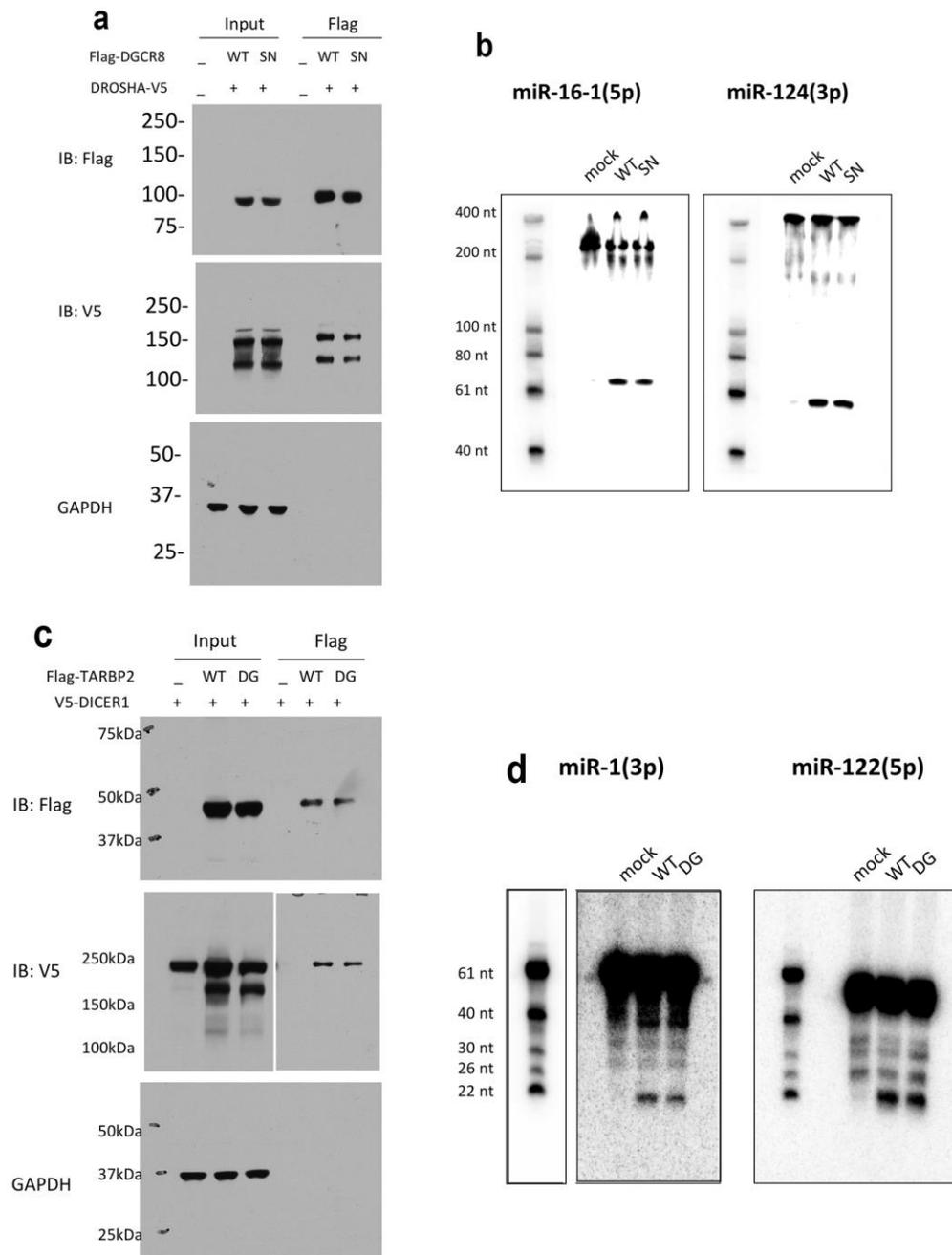


Supplementary Figure 1. Expression and immunoprecipitation of wild-type and mutant DICER1 (a) and DROSHA (b) proteins used for *in vitro* processing assays.



Supplementary Figure 2. *In vitro* processing activity of wild-type and mutant DGCR8 and TARBP2. **a**, Cells were co-transfected with wild-type or mutant DGCR8 harboring the S702N (SN) substitution together with V5-tagged DROSHA. Western blots show equivalent expression and immunoprecipitation of wild-type and mutant proteins. **b**, *In vitro* processing assays demonstrate equivalent activity of wild-type and DGCR8 S702N proteins. **c**, Cells were co-transfected with wild-type or mutant TARBP2 harboring the D221G (DG) substitution together with V5-tagged DICER1. Western blots show equivalent expression and immunoprecipitation of wild-type and mutant proteins. **d**, *In vitro* processing assays demonstrate equivalent activity of wild-type and TARBP2 D221G proteins.

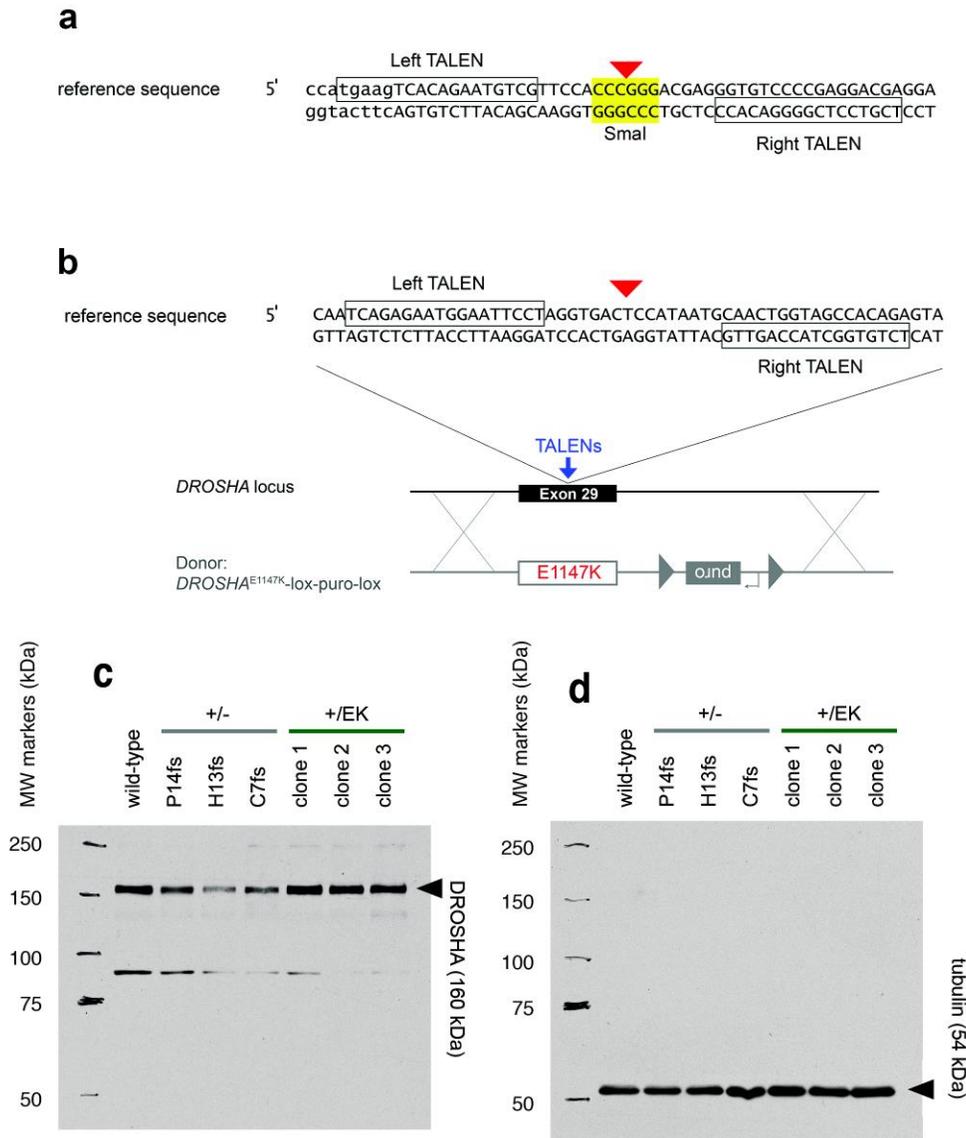
CMCW7 *DROSHA* 3451G>T

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CMCW7_2 ATGGAATTCCTAGGTGACTCCATAATGCAAC
CMCW7_3 ATGGAATTCCTAGGTGACTCCATAATGCAAC
CMCW7_4 ATGGAATTCCTAGGTGACTCCATAATGCAAC
CMCW7_5 ATGGAATTCCTAGGTGACTCCATAATGCAAC
CMCW7_6 ATGGAATTCCTAGGTGACTCCATAATGCAAC
CMCW7_7 ATGGAATTCCTAGGTGACTCCATAATGCAAC
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DROSHA Ref Seq ATGGAATTCCTAGGTGACTCCATAATGCAAC
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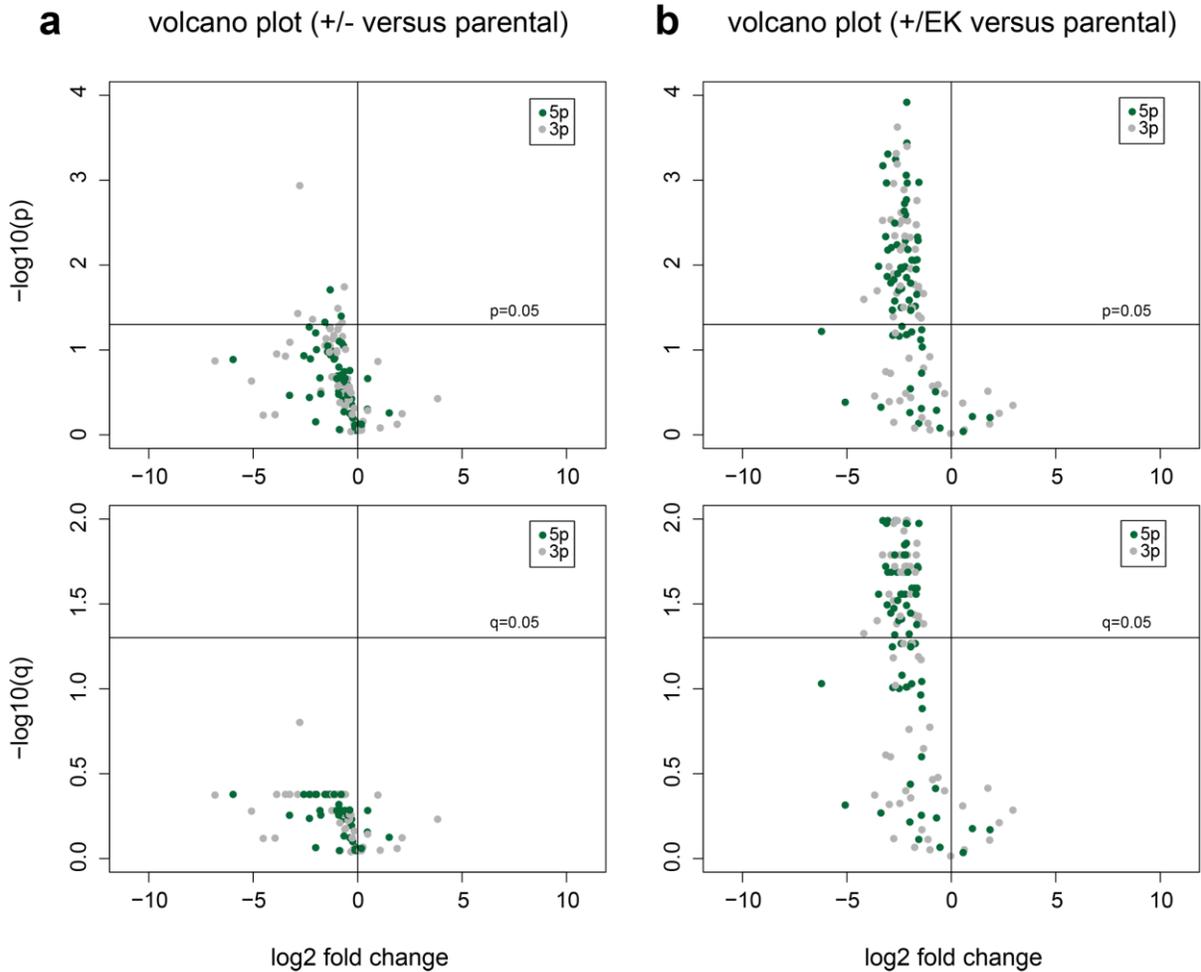
CMCW15 *DROSHA* 3439G>A

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CMCW15_4 CACAATCAGAGAATGGAATTCCTAGGTGACT
CMCW15_5 CACAATCAGAGAATGGAATTCCTAGGTGACT
CMCW15_6 CACAATCAGAGAATGGAATTCCTAGGTGACT
CMCW15_7 CACAATCAGAGAATGGAATTCCTAGGTGACT
CMCW15_8 CACAATCAGAGAATGGAATTCCTAGGTGACT
CMCW15_9 CACAATCAGAGAATGGAATTCCTAGGTGACT
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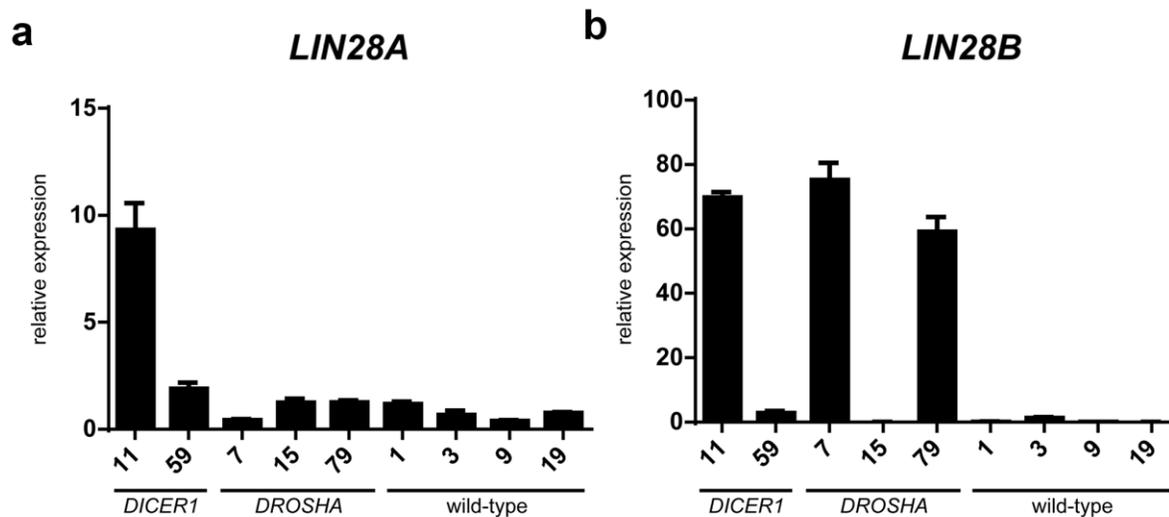
Supplementary Figure 3. Sanger sequencing of RT-PCR products from *DROSHA* mutant tumors demonstrating continued expression of wildtype alleles.



Supplementary Figure 4. Modeling of *DROSHA* mutations using TALENs. **a**, Schematic of TALEN strategy for targeting second coding exon. TALEN cleavage and subsequent introduction of Indels causes destruction of SmaI site. **b**, Schematic of TALEN strategy for targeting region surrounding codon 1147. TALEN cleavage induces homologous recombination. The exogenous repair template includes silent mutations to prevent re-cleavage by the TALEN, the missense E1147K mutation, and a floxed puromycin selection cassette. **c**, full version of *DROSHA* immunoblot from Figure 4b. The band at approximately 85 kDa represents non-specific immunoreactivity. **d**, full version of alpha-tubulin immunoblot from Figure 4b.



Supplementary Figure 5. Volcano plots of microRNA expression in mutant clones. **a**, *DROSHA*^{+/-}. **b**, *DROSHA*^{+/E1147K}. Green dots are 5' miRNAs and gray dots are 3' miRNAs. *p* values (upper plots) calculated by two-tailed t test; *q* values (lower plots) represent correction for false-discovery rate using Benjamini-Hochberg method. Horizontal line drawn at 0.05 in each plot.



Supplementary Figure 6. Expression of **a**, *LIN28A* and **b**, *LIN28B* in Wilms tumor samples, measured by qPCR. Wilms tumor samples listed by CMCW number, and grouped by mutation status (mutation in *DICER1*, *DROSHA*, or neither). Bars represent standard error from three technical replicates of each sample.

Supplementary Note 1: Sequences of TALENs

Amino acid sequences of transcription activator-like effector (TALE) repeat arrays used for genome editing in HCT116 cells. Repeat variable domains (RVDs) underlined.

TALEN Exon2 Left

MVDLRTLGYSSQQQEKIKPKVSRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQD
MIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIARKGGVTAVEA
VHAWRNALTGAPLNLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIG
GKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVVA
IANNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLT
PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLC
QAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQR
LLPVLCQDHGLTPEQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQA
LETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNG
GGKQALETVQRLLPVLCQDHGLTPEQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPDQV
VAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDHG
LTPEQVVAIANNNGGRPALE

TALEN Exon2 Right

MVDLRTLGYSSQQQEKIKPKVSRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQD
MIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIARKGGVTAVEA

VHAWRNALTGAPLNLTDPQVVAIASHHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIANNN
GGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQV
VAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHHDGGKQALETVQRLLPVLCQDHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHHDGGKQALETVQRLLPV
LCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALET
VQRLLPVLCQDHGLTPEQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPDQVVAIANNN
GGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGGKQALETVQRLLPVLCQDHGLTPDQVVA
IASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGGKQALETVQRLLPVLCQAHGLTP
DQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGRPALE

TALen E1147 Left

MVDLRTLGYSSQQQEKIKPKVRSSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQD
MIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAGRGGVTAVEA
VHAWRNALTGAPLNLTDPQVVAIASHHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIG
GKQALETVQRLLPVLCQAHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQVV
AIASNIGGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGL
TPEQVVAIASNIGGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGGKQALETVQRLLPVLC
QAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQDH
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VLCQAHGLTPDQVVAIASNIGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGGKQALET
QRLLPVLCQDHGLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGK
QALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIA
SHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGRPALE

TALen E1147 Right

MVDLRTLGYSSQQQEKIKPKVRSSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQD
MIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAGRGGVTAVEA
VHAWRNALTGAPLNLTDPQVVAIASHHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNG
GGKQALETVQRLLPVLCQAHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQV
VAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQDH
GLTPEQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLP
VLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNIGGGKQALET
VQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGG
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